NEW INJECTABLE FORMULATIONS CONTAINING RAMOPLANIN

The present invention relates to a new injectable formulation of ramoplanin or a compound of the ramoplanin family. More particularly, the injectable formulations of the invention are particularly suitable for intravenous (i.v.) administration.

Ramoplanin (INN) is a known member of the cyclic peptide antibiotics more precisely known as glycolipodepsipeptides which has been described in US 4,303,646 and 4,328,316. Originally it has been named antibiotic A 16686. It is a complex substance whose separate factors A_1, A_2 and A_3 have been described in US 4,427,656.

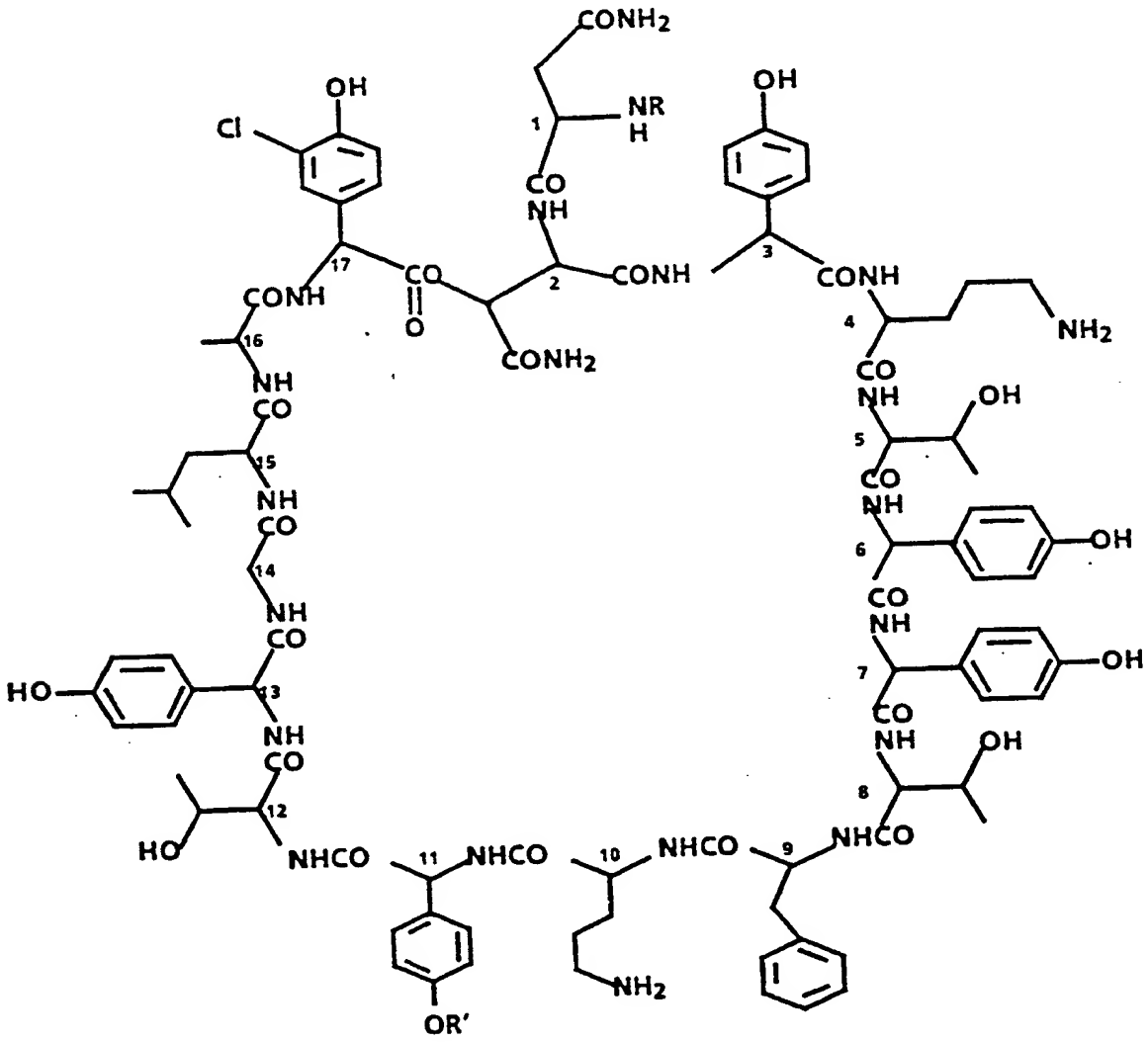
Ramoplanin factors A'_1, A'_2 and A'_3 have been described in EP-B-318680, the aglycones of any of the above factors have been described in US 5,491,128 while the tetra hydrogenated derivatives of any of the above factors have been described in US 5,108,988. A method for selectively increasing the ratio of single major components A_2 and A_3 is described in EP 0259780. All the above mentioned patents are incorporated herein by reference.

The structure of ramoplanin and its factors and derivatives have been described in several articles and publications, see R. Ciabatti et al., J. Antib. 1989, 254-267, J. K. Kettenring et al., J. Antiob. 1989, 268-275, R. Ciabatti and B. Cavalleri, Bioactive Metabolites from Microorganisms, Elsevier Science

Publisher, 1989, 205-219 and M. Kurz and W. Guba, Biochemistry 1996, 35, 12570-12575.

N.J. Skelton et al. in J. Am. Chem. Soc. 1991, 113, 7522-7530 describe another member of this family, which they call Ramoplanose.

These compounds can be represented by the following formula (Formula I):



FORMULA I

wherein:

R represents -CO-CH=CH-CH=CH-CH_2-CH_2-CH_3,
-CO-CH=CH-CH=CH-CH_2-$CH(CH_3)_2$,
-CO-CH=CH-CH=CH-CH_2-CH_2-$CH(CH_3)_2$,
-CO-CH_2-CH_2-CH_2-CH_2-CH_2-CH_2-CH_3,
-CO-CH_2-CH_2-CH_2-CH_2-CH_2-$CH(CH_3)_2$ or
-CO-CH_2-CH_2-CH_2-CH_2-CH_2-CH_2-$CH(CH_3)_2$

R' represents alpha-D-mannopyranosyl or 2-O-alpha-D-mannopyranosyl-alpha-D-mannopyranosil, or

R' represents 2,3-O-di[alpha-D-mannopyranosyl]-D-mannopyranosyl when R represents -CO-CH=CH-CH=CH-CH_2-$CH(CH_3)_2$,

a pharmaceutically acceptable acid addition salt thereof, or a mixture thereof in any proportion.

The configuration of the double bonds of the unsaturated moieties reported above in the definition of R have been found to be 2(E) or *cis* and 4(Z) or *trans* in the literature reported above.

The following table specifies the meanings for R and R' of the single factors or derivatives with reference to the above formula:

Factor	R	R'
A_1	-CO-CH=CH-CH=CH-CH_2-CH_2-CH_3	2-O-alpha-D-manno-pyranosyl-alpha-D-mannopyranosyl
A_2	-CO-CH=CH-CH=CH-CH_2-$CH(CH_3)_2$	2-O-alpha-D-manno-pyranosyl-alpha-D-mannopyranosyl
A_3	-CO-CH=CH-CH=CH-CH_2-CH_2-$CH(CH_3)_2$	2-O-alpha-D-manno-pyranosyl-alpha-D-mannopyranosyl
A'_1	-CO-CH=CH-CH=CH-CH_2-CH_2-CH_3	Alpha-D-mannopyranosyl
A'_2	-CO-CH=CH-CH=CH-CH_2-$CH(CH_3)_2$	Alpha-D-mannopyranosyl
A'_3	-CO-CH=CH-CH=CH-CH_2-CH_2-$CH(CH_3)_2$	Alpha-D-mannopyranosyl

The aglycones correspond to the compounds reported above wherein R' represents hydrogen while the tetrahydrogenate derivatives correspond to the compounds reported above wherein the mojety R is fully hydrogenated.

Ramoplanose is reported to correspond to "factor A_2" wherein R' represents 2,3-O-di[alpha-D-mannopyranosyl]-D-mannopyranosyl.

In the following description and claims, the term "ramoplanin" refer to a ramoplanin complex wherein factor A_2 is the major component, with a small amounts of factors A'_2, A_1, A'_1, A_3, A'_3 and other related substances accounting for the remainder of this active ingredient.

Particularly preferred is "ramoplanin" wherein factor A_2 represents at least 75% of the active ingredient.

"A member of the ramoplanin family" refers to any of the compounds reported above that are represented by Formula I, any salt or any mixture thereof in any proportion.

Ramoplanin as well as any members of the ramoplanin family are unsuitable for i.v. administration because of drawbacks such as swelling and progressive necrotization at the site of injection, and haemolysis as revealed by urine discoloration.

The formulations of the invention contain ramoplanin or a member of the ramoplanin family in admixture with a fat emulsion product for intravenous administration.

In general, for i.v. administration purposes according to this invention, it is suitable to utilize liquid compositions wherein ramoplanin or a member of ramoplanin family is present in concentration from 1 to 20 mg/ml, preferably, from 1.5 to 15 mg/ml, most preferably, from about 3 to about 5 mg/ml.

In the current description and claims the expressions "fat emulsion product for intravenous injection" or "fat emulsion product" identify any of those fat emulsion products suitable for intravenous administration via a peripheral vein or by a central venous infusion that are currently used mainly to assure calories intake when parenteral nutrition is required. Examples of these substances are for instance reported in US Pharmacopeia, Martindale, The Extra Pharmacopeia (31st edition, 1996, page 1377) or VIDAL

1996, page 814. The above expressions include also those emulsions used as colloidal drug carriers, examples of which are reported in the book "Submicron Emulsions in Drug Targeting and Delivery" edited by S. Benita, Horwood Academic Publishers, 1998, at pages 119-122. All above cited publications are incorpored hereby by reference.

The above said fat emulsion products are largely based on an oil phase stabilized by emulsifiers, like phospholipids, poloxamers or other polyoxyethylene derivatives such as, for instance, polysorbates or polyoxyethylene castor oil.

~~Typically, a fat emulsion product suitable for~~ preparing a formulation of the invention comprises an oil phase (usually 2-40%, preferably, 5-25% weight/vol), preferably consisting of vegetable oils such as soybean oil, safflower oil and cottonseed oil, emulsifiers (usually 0.2-5%, preferably, 0.5-2% weight/vol), preferably based on phospholipids of egg source such as, egg lecithin or soybean lecithin, and additives as osmotic agents such as glycerol, sorbitol ~~and xylitol.~~

~~These fat emulsion products, as commercially~~ available, are emulsions comprising the above mentioned oil phase, emulsifiers and additives dispersed in water for injection and the oil phase is generally present in the emulsion in a percentage (weight/vol) of 5 to 25%. For preparing the i.v. administrable formulation of this invention, the fat emulsions product may be used as such or diluted with saline or water for injection ~~added with an osmotic agent (e.g. glucose) to decrease~~

~~the oil phase concentration to a lower value and, at the same time, maintaining the desired osmolarity.~~

In general, if the concentration of ramoplanin or a member of ramoplanin family in the formulation is low, it is possible to lower the percentage of the oil phase in said i.v. formulation.

~~For instance, with ramoplanin concentrations of~~ about 10 mg/ml, the percentage of the oil phase in the i.v. formulations of the invention may range between 4 and 40% (weight/vol) although are preferred those i.v. fat emulsions wherein the oil phase is between 4 and 25%, and, more preferably, between 8 and 18%, with the range 8-10%being currently the most preferred ~~concentration.~~

With ramoplanin concentrations of about 1 mg/ml the percentage of the oil phase in the i.v. formulation can be lowered to a range between 0.2 and 10% (weight/vol).

Generally, the osmolarity of the final i.v. formulation is between 250 and 300 mOsm/L, while the value of the pH must be compatible with the stability of ramoplanin (or a member of the ramoplanin family), and, therefore, usually, it should not be higher than 8.

As known in the art, particle size of the emulsion needs to be controlled for a proper i.v. administration, and this is accomplished through the conventional preparation and final formulation procedures.

Examples of fat emulsion products that can be conveniently used according to the present invention are those listed at page 120 of the above cited book: "Submicron Emulsion in Drug Targeting and Delivery" where the oil phase consists of soybean oil, cottonseed oil, safflower oil or mixture thereof.

Soybean oil, cottonseed oil and safflower oil contain long chain fatty acids comprising mainly linoleic acid, oleic acid, palmitic acid, linolenic acid and stearic acid, essentially in the form of triglycerides.

Soybean oil, cottonseed oil and safflower oil can be totally or in part substituted by any mixtures of the above fatty acids in the form of triglycerides having a percent (weight/weight) composition substantially similar to that of the above oils or their mixtures. Moreover, part of the above mentioned vegetable oils or long chain fatty acids triglycerides may be substituted by medium chain (C_6-C_{12}) triglycerides.

Typically, the fat emulsion product used for the preparation of the i.v. formulations of this invention contains an oil phase in a range from 2 to 40 percent (weight/vol), preferably, from 5 to 25 percent, more preferably from 7 to 20 percent, emulsifier(s) in a range from 0.2 to 5 percent (weight/vol), preferably, from 0.6 to 2 percent more preferably from 0.5 to 1.5 percent, and the additive is in an amount suitable to control osmolarity, preferably, in a range from 1.5 to 5 percent (weight/vol), more preferably preferably from 2 to 3 percent.

In said oil phase consisting of soybean oil, cottonseed oil or safflower oil or mixture thereof, or in the fatty acids mixtures which may substitute totally or in part the above oils, the fatty acids triglycerides are usually present in the following percent (weight/weight) proportion indicated between brackets: linoleic acid (40-70%), oleic acid (15-30%), palmitic acid (5-15%), linolenic acid (3-12%), stearic acid (2-6%).

As indicated above, for the preparation of the i.v. formulations of this invention, the above said fat emulsion products are used as such or are diluted in a isoosmotic water solution for injection to a concentration of the oil phase in the final composition that is at least 0.2% (weight/vol), normally, depending on the concentration of ramoplain or a member of ramoplanin family which is present in the final composition.

According to a preferred embodiment of this invention, those fat emulsion products that are currently available under the trade names Intralipid®, Liposyn® and Lipofundin® may be utilized. For instance, Intralipid® (Kabi Vitrum/Pharmacia) and Liposyn® II and Liposyn® III (Abbott), have composition and physico-chemical properties as reported below:

Table I. Composition and characteristics of Various Intravenous Fat Emulsions

Compon nts or Characteristics	Intralipid® (Kabi-Vitrum/Pharmacia)			Liposyn® II (Abbott)			Liposyn® III (Abbott)		
Soybean oil (w/vol)	10%		20%	5%		10%	10%		20%
Safflower oil (w/vol)	--		--	5%		10%	--		--
Egg yolk phospholipids (w/vol)	1.2%		1.2%	1.2%		1.2%	1.2%		1.2%
Glycerol (w/vol)	2.25%		2.25%	2.5%		2.5%	2.5%		2.5%
Water for injection	QS		QS	QS		QS	QS		QS
Fatty acids composition of vegetable oils (w/w)									
Linoleic acid		50%			65.8%			54.5 %	
Oleic acid		26%			17.7%			22.4 %	
Palmitic acid		10%			8.8%			10.5 %	
Linolenic acid		9%			4.2%			8.3%	
Stearic acid		3.5%			3.4%			4.2%	
Osmolarity (mOsm/L)	260		268	276		258	284		292
Approximate pH	8		8	8		8.3	8.3		8.3
Fat particle size (μm)	0.5		0.5	0.4		0.4	0.4		0.4
Caloric value (cal/ml)	1.1		2.0	1.1		2.0	1.1		2.0
Size (ml)	50, 100		50, 100	25, 50		25, 50	100,200		200,500
	250,500		250,500	100,200		200,500	500		
				500					

As stated above, in the formulations according to this invention ramoplanin or a member of the ramoplanin family as defined above is generally present in the compositions of the invention in an amount of 1 to 20 mg/ml, with a range of 1.5 to 15 mg/ml being currently preferred, and a range from about 3 to about 5 mg/ml being the most preferred one.

Typically, the composition of the invention is a composition wherein the oil phase in the final fat emulsion is between 0.2 and 40%(weight/vol), with a range 4-25% being preferred, a range 8-18% being more preferred and with the range 8-10% being currently most preferred. However, as mentioned above, the proportion of the oil phase may be adjusted to the one of the antibiotic and to lower amounts of ramoplanin may correspond lower amounts of oil phase in the composition

Experiments with representative examples of the compositions of the invention have shown a good tolerability at the site of injection, in particular in comparison with the effects of conventional i.v. preparations of the same active principle.

~~The results of a first set of tolerability studies~~ Data in representative examples of formulations of the invention in rats at a concentration of ramoplanin of 10 mg/ml (dose 20 mg/kg, administration volume 2 ml/kg), in comparison with a conventional i.v. formulation of the same active principle, are ~~summarized in the following~~.

More particularly, ramoplanin in a conventional acqueous vehicle (0.9% saline) or in the formulations of the invention wherein the proportion of the oil phase in the total formulation is between 2 and 8% (weight/vol) is administered to rats (3-5 animal/group) at a dose of 20 mg/kg (drug concentration 10 mg/ml). The administered volume is 2 ml/kg, according to the animal weight on the day of administration, and the injection speed is 0.1 ml/sec. The intravenous administration is into the caudal vein. Treatments are planned for three days at 24 hours intervals. Control rats receive either 0,9% saline or an equivalent volume of Intralipid® 10%. Behavior and physical appearance are observed frequently the day of dosing. Urine appearance is also recorded within 3 h after each daily treatment. Rats are sacrified 24 h after the last treatment. The results of these experiments are summarized in Table II.

Table II. Urine appearance, gross pathology at the injection site and clinical observations of rats treated with ramoplanin formulated with Intralipid® or in a conventional aqueous vehicle (0.9% saline)

Groups	No. Animals	Saline (w/vol)	Intralipid® (a) (w/vol)	Ramoplanin concentration	Urine Appearance (b)	Gross Pathology at the injection site (c)
A	3	0.9%	--	--	Normal	Normal
B	4	0.9%	--	10 mg/ml (d)	Red-brown	Dark, discolored tails
C	3	--	10%		Normal	Normal
D	5	--	8%	10 mg/ml	Normal	Normal
E	5	--	4%	10 mg/ml	Normal	Normal
F	5	--	2%	10 mg/ml	Red-brown	Dark, discolored tails

(a) In water for injection, q.s. 100%

(b) Visual examination performed within 2-3 h after each scheduled treatment

(c) Examinations performed at the end of the three scheduled treatments

(d) Corresponding to a dose of 20 mg/kg

Treatments with ramoplanin at a concentration of 10 mg/ml in conventional aqueous vehicle or in formulation with 2% (weight/vol) of oil phase caused darkness or discoloration at the injection site (tail). In contrast, treatment with the formulations of the invention wherein the oil phase was 4% (weight/vol) or higher was well tolerated. Tails did not show any sign of necrotic inflammation.

After the immediate postdose period of each treatment (3 h) with the formulations of the invention wherein the oil phase was 4% (weight/vol) or higher, the urine appeared light straw to dark yellow in colour. In contrast, rats given a 2% (weight/vol) oil phase formulation or ramoplanin in conventional aqueous vehicle developed red to red-brown urine, within the same postdose period.

~~A second set of experiments~~ was carried out to determine tolerability of the formulation of the invention according to the same procedure described above but administering a dose corresponding to 10 mg/kg instead of 20 mg/kg to several groups of three rats for 3 days at 24 hours intervals with. The concentration of ramoplanin in the formulation was 1 mg/ml instead of 10 mg/ml and the volume of the formulation administered to each rat was 10 ml/kg instead of 2 ml/kg. The Intralipid® fat emulsion product was added in several different proportion as represented in the following Table III where the same parameters considered in Table II are reported. The ~~rats were killed 24 h after the last treatment.~~

Table III: Urine appearance, gross pathology at the injection site and clinical observations of rats treated with ramoplanin formulated with Intralipid® or in a conventional aqueous vehicle (0.9% saline)

Groups	No. Animals	Saline (w/vol)	Intralipid® (w/vol) (a)	Ramoplanin concentration	Urine Appearance (b)	Gross Pathology at the injection site (c)
A	3	0.9%	--	1 mg/ml **(d)**	Red-brown	Dark tails (2/3)
B	3		9%	1 mg/ml	Normal	Normal
C	3	--	1%		Normal	Normal
D	3	--	0.5%	1 mg/ml	Normal	Normal
E	3	--	0.2%	1 mg/ml	Normal	Normal
F	3	--	0.1%	1 mg/ml	Red-brown	Discolored tails (3/3)

(a) In water for injection, q.s. 100%

(b) Visual examination performed within 3 h after each scheduled treatment

(c) Examinations performed at the end of the three scheduled treatments

(d) Corresponding to a dose of 10 mg/kg

The above data show that ramoplanin at a concentration of 1 mg/ml can be safely administered intravenously to experimental animals at a dosage of 10 mg/kg when the drug is appropriately formulated according to this invention in emulsion compositions containing Intralipid® in such amount that the oil phase is at least 0.2 per cent (w/vol) of the total formulation.

The effectiveness of representative examples of the compositions of the invention in experimental animal models can be demonstrated in several acute septicemia experiments in immunocompetent or neutropenic mice and in experiments of endocarditis and pneumococcal lobar pneumonia in rats.

Experimental septicemia is induced by inoculating intraperitoneally (5-6 animal/dose/treatment group) a bacterial suspension of either a clinical isolate of a methicillin resistant staphylococcus (*Staph. aureus* L613) or streptococcus strain (*Strep. pneumonia* L 44) in immunocompetent mice or a clinically isolated glycopeptide resistant enterococcus strain (*Ent. faecium* L569) in neutropenic mice. Immunocompetent mice are male and female CD_1 mice (Charles River Labs., Calco, Italy) weighting 18-22 g while neutropenic mice are 6-8 weeks old female NMRI mice (Iffa Credo, France).

Untreated animals die within 24-72 h after infection. Antibiotic treatment begins within 10 min after injection. Ramoplanin at various concentration is administered intravenously in conventional aqueous

vehicle or in the formulation of the invention in 8% (weight/vol) oil phase fat emulsion. Gentamicin, vancomycin, teicoplanin and rifampicin can be included as comparator drugs. The 50% effective dose (ED_{50}) and 95% confidence limits are calculated by the Spearman-Karber method from the percentage of animal surviving at day 10.

The animals are treated twice, first 10 min from infection and then 24 h later.

~~When the gentamicin or vancomycin are employed as~~ comparators, they are administered subcutaneously and the second shot is given 5 h after infection. Rifampicin and teicoplanin are administered ~~subcutaneously in single dose 10 min after infection.~~

Results of experiments conducted as described above are reported in the following table:

Table IV. ED_{50} of ramoplanin in experimental septicemia in mice.

Strain (animal)	**Formulation**	**ED_{50} mg/kg/dose (95% confidence limits)**
VanA *Ent.faecium* L 569 (neutropenic mice)[a]	Ramoplanin in 0.9% saline	**5.1 (d)**
	Ramoplanin in 8% Intralipid®	**1.7 (1.4-2.0)**
Staph. aureus L 613 (immunocompetent mice)[b]	Ramoplanin in 0.9% saline	**4.3 (3.1-6.0)**
	Ramoplanin in 8% intralipid®	**5.1 (3.9-6.5)**
Strep. pneumonia L 44 (immunocompetent mice)[c]	Ramoplanin in 0.9% saline	**0.06 (d)**
	Ramoplanin in 8% Intralipid®	**0.06 (d)**

[a] ED_{50} of comparators were as follows: gentamicin 50.6 (37.3-68.7), rifampicin 1.2 (0.9-1.5), vancomycin > 90%.

[b] ED_{50} of comparator (teicoplanin) was 5.4 (4.3-6.9).

[c] ED_{50} of comparator (teicoplanin) was 0.79 (0.65-0.96).

[d] Confidence limit could not be calculated because survival was either 0 or 100% in each treatment group.

Endocarditis experiments can be performed in experiment animals (rats) with isolates of staphylococci or enterococci. A polyethylene catheter is inserted through the aortic valve into the left ventricle of the animal via the right carotid artery. Two days later, the animals are infected i.v. Treatment begins the day after infection and continues for a total of 5 days. Surviving animals are killed on day 7 after infection. The hearts of all animals are homogenized and processed to determine bacterial load, that is expected to be substantially reduced in the treatment group receiving the formulations of the invention, in comparison with untreated controls.

Pneumonia experiments can be performed in both immunocompetent and neutropenic rats with e.g. a clinically isolated penicillin-resistant *Strep. pneumoniae* strain. Anesthetized animals are infected by surgical intrabronchial instillation via intratracheal intubation, with a 40 µl inoculum containing approximately 10^6 to 10^7 $\log_{10}$ CFU (colony forming units) of *Strep.pneumoniae* and are allowed to recover. Therapy is initiated 12 h after infection and continued for a total of three days. Surviving animals are killed on day 4 after infection. The lungs of all animals are homogenized and processed to determine bacterial load, that is expected to be substantially reduced in the treatment group receiving the formulations of the invention, in comparison with the untreated control.

The results reported above show that the formulations of the invention are in general well

tolerated, in particular at the injection site, as demonstrated by the absence of necrotic inflammation and urine discoloration.

The results indicate that the delivered drug is effective in treating infections caused also by multiresistant microorganisms.

The formulations of the invention therefore can be effectively administered to a patient in need thereof to control or cure infections sustained by microorganisms that are known to be susceptible to ramoplanin or an antibiotic of the ramoplanin family.

Particularly preferred is the use of the formulations of the invention in antibiotic treatment of serious Gram positive infections such as bacteremia, endocarditis and pneumonia. In particular the use of the formulations of the invention is expecially suitable for systemic treatment of severe infections caused by Gram positive resistant or multiresistant microorganisms, such as coagulase-positive and negative staphylococci, penicillin resistant streptococci or glycopeptide resistant enterococci.

In the present disclosure, the term "patient" is intended to refer to warm blooded animals such as rodents, felines, equines, bovids, and primates, including humans. Preferred as "patients" according to the invention, in addition to humans, are pet and farm animals.

An example of dosage range of ramoplanin or a member of the ramoplanin family that can be administered through formulation of the invention, that is predicted to be effective for human therapy, is preferably between 0.5 and 1 g/die, while a preferred formulation contains about between 1 and 20 mg/ml, preferably, between 1.5 and 15 mg/ml, most preferably between about 3 to about 5 mg/ml of ramoplanin or a member of ramoplanin family.

Particularly preferred is the use of the formulations of the invention in severe enterococcal infections, particularly those attributable to vancomycin-resistant strains, for which no really effective treatment is currently available (see for instance M.B. Edmond et al., Clinical Infectious Diseases, 1996; 23: 1234-1239) as well as infections wherein penicillin-resistant streptococci are present.

In such treatments, the formulation of the invention is preferably employed as a slow infusion by a central vein.

The formulations of the invention are prepared according to the conventional techniques, on the basis of the present disclosure. The pH of the final preparation is lower than 7 and preferably between 4 and 6.5, with a pH between 5.5 and 6.5 being currently most preferred.

If necessary the pH of the final formulation is adjusted to the desired value by the known procedures.

The ramoplanin (or a member of ramoplanin family) i.v. formulation of this invention can be in the form of a ready to use dosage form containing both the antibiotic and the fat emulsion product or can be in the form of a kit comprising separate packagings or containers containing ramoplanin (or a member of ramoplanin family), and the fat emulsion product for constitution of said i.v. formulation when use is needed. In particular, said kit may consist of vials or similar containers containing the dose of lyophylized sterile antibiotic, ampuls containing water for injection in amount sufficient to dissolve the antibiotic and bottles containing the sterile fat emulsion product in amount appropriate for constituting the desired i.v. formulation.

Examples of specific formulations of the invention and formulation procedures are reported below.

Table V. Formulations of ramoplanin (10 mg/ml) in varying dilutions of Intralipid®.

	10% (w/vol) Intralipid (ml)	5% Glucose (w/vol)	50 mg/ml Ramoplanin (ml)	Intralipid® / Ramoplanin
A	8	--	2	8% (w/vol) / 10 mg/ml
B	4	4	2	4% (w/vol) / 10 mg/ml
C	2	6	2	2% (w/vol) / 10 mg/ml

Operatively, to 10% Intralipid® (Pharmacia), under moderate stirring, the glucose solution is slowly added followed by the ramoplanin solution.

The solution of ramoplanin in distilled water is prepared by dissolving 562 mg of ramoplanin (89% potency determined by a HPLC assay) in distilled water (5 ml) and then bringing to the final volume (10 ml).

Table VI. Formulations of ramoplanin (1 mg/ml) in varying dilutions of Intralipid®.

	10% (w/vol) Intralipid (ml)	0.9% Saline (w/vol)	10 mg/ml Ramoplanin (ml)	Intralipid® / Ramoplanin
D	9	--	1	9% / 1 mg/ml
E	1	8	1	1% / 1 mg/ml
F	0.5	8.5	1	0.5% / 1 mg/ml
G	0.2	8.8	1	0.2% / 1 mg/ml

A solution of ramoplanin 10 mg/ml of activity was prepared in NaCl 0.9% (w/vol). The solution was sterilized by filtration with 0.22 µm pore-size filters.

1 ml of the ramoplanin solution was added to an aliquot of Intralipid® diluted to the desired concentration by slow addition of the appropriate volume of 0.9% NaCl. The mixture was vigorously shaken to obtain a homogeneous dissolution in the fat emulsion.